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Effects of quercetin on oxidative stress and memory retrieval in kindled rats

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ABSTRACT

Flavonoids are a class of polyphenolic compounds present in fruits and vegetables. Several studies have demonstrated a relationship between the consumption of flavonoid-rich diets and the prevention of human diseases including neurodegenerative disorders. Thus, we assessed the effect of quercetin (3,3′,4′,5,7-pentahydroxyflavone) on oxidative stress and memory retrieval using a step-through passive avoidance task in kindled rats. Quercetin (25, 50, and 100 mg/kg) was administered intraperitoneally (i.p.) before pentylenetetrazole (PTZ) every other day prior to the training. Retention tests were performed to assess memory in rats. Compared to control, pretreatment with 50 mg/kg of quercetin could attenuate seizure severity from the beginning of the kindling experiment by lowering the mean seizure stages. Moreover, quercetin 50 mg/kg significantly increased the step-through latency of the passive avoidance response compared to the control in the retention test. Malondialdehyde (MDA) levels were significantly increased in the quercetin groups compared to the PTZ group in the hippocampus and cerebral cortex following PTZ kindling. In the quercetin groups, higher sulfhydryl (SH) contents were not observed compared to the PTZ group. These results indicate that quercetin at a specific dose results in decreased seizure severity during kindling and performance improvement in a passive avoidance task in kindled rats. All doses of quercetin led to increased oxidative stress in the hippocampi and cerebral cortices of kindled rats.

1. Introduction

Pentylenetetrazole (PTZ), a selective blocker of the chloride channel coupled to the $GABA_A$ receptor complex, is the most popular chemoconvulsant used for evaluating antiepileptic drugs [1,2]. Kindling induced by the convulsant PTZ represents a model of primary generalized epilepsy [3]. The PTZ kindling model provides a useful model of postseizure dysfunction, which can serve as a screen for potential treatments for those cognitive and emotional deficits often observed in human epilepsy [4]. The effects of epilepsy on cognition have been reviewed in several papers [5,6]. Moreover, a mild decline in intellectual performance in children and adults with epilepsy has been suggested [5]. Patients with localized epilepsies generally have deficits in the cognitive functions controlled by respective areas, such as memory impairment in temporal lobe epilepsy (TLE) [6]. There are several pieces of evidence that indicate that PTZ kindling impairs shuttle-box performance [7–11].

Quercetin is a flavonoid (3,3′,4′,5,7-pentahydroxyflavone) found in a variety of fruits and vegetables including apples, citrus fruits, berries, bulbs, cereal grains, onions, legumes, and tea [12,13]. Quercetin has several pharmacological properties, including antioxidant, antiinflammatory, and hepatoprotective effects [12,14–18]. A high dosage of quercetin is needed to achieve good results since quercetin undergoes first-pass metabolism [19]. It has the ability to penetrate the blood–brain barrier (BBB), and in some studies, the proportion of its passage is estimated to be as high as 65.54% [20]. Quercetin metabolites and glycosides seem to be less neuroprotective and penetrate the BBB less efficiently than aglycone [21]. There are several studies about the inhibitory role of quercetin on lipid peroxidation in different behavioral studies [22–25]. Thus, in this study, we focused on the possible effects of quercetin on oxidative stress and memory retrieval in kindled rats. Malondialdehyde (MDA) and sulfhydryl (SH) groups were measured as indicators for lipid peroxidation and oxidative stress markers.

2. Material and methods

2.1. Animals

Male Wistar rats (200–250 g) were obtained from Razi Institute (Karaj, Iran) and housed in groups of four per cage under standard laboratory conditions. They were kept at a constant room temperature

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(21 ± 2 °C) under a normal 12L:12D regime with free access to food and water. All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) in such a way as to minimize the number of animals and their suffering.

2.2. Drugs

Quercetin and PTZ were purchased from Sigma (St. Louis, MO, USA). Other drugs in this investigation were xylazine (Loughrea, Co. Galway, Ireland) and ketamine (Rotexmedica, GmbH, Germany). Quercetin and PTZ were dissolved in saline. 2,2′-Dinitro-5,5′-dithiodibenzoic acid (DTNB), 2-thiobarbituric acid (TBA), Tris (Trizma® base), sodium ethylenediaminetetraacetic acid (Na EDTA), methanol, trichloroacetic acid (TCA), potassium chloride (KCL), and hydrochloric acid (HCL) were purchased from Merck (Darmstadt, Germany).

2.3. Kindling procedure by PTZ

Rats were divided into four groups of 10 animals each. In the control group, saline was injected 30 min before administration of PTZ every other day (35 mg/kg, i.p., 15 injections total).

After each injection, the animals were placed individually in plastic boxes, and their convulsive behavior was recorded for 30 min; behavioral changes were graded according to the criteria described by Racine [26] as modified by Xie et al. [27]: Stage 0: no response; Stage 1: hyperactivity and vibrissae twitching; Stage 2: head nodding, head clonus, and myoclonic jerk; Stage 3: unilateral forelimb clonus; Stage 4: rearing with bilateral forelimb clonus; and Stage 5: generalized tonic–clonic seizures (GTCSs) with loss of postural control.

In the remaining three groups, 25, 50, or 100 mg/kg of quercetin was given 30 min before administration of PTZ every other day (35 mg/kg, i.p., 15 injections total).

For memory testing, a group of animals with unimpaired memory was used; they had been injected with saline instead of PTZ. This group was used to elucidate the effects of quercetin on memory under baseline conditions, compared with levels that were lower or higher than the baseline.

2.4. Passive avoidance apparatus

The passive avoidance apparatus comprised a learning box consisting of a light (white) and a dark (black) compartment 20 × 20 × 30 cm each. A guillotine door opening (6 × 6 cm) was made on the floor in the center of the partition between the two compartments. Stainless steel grids (5 mm in diameter) were placed at 1-cm intervals (distance between the centers of the grids) on the floor of the dark compartment to produce a foot shock.

All rats were allowed to habituate to the experimental room for at least 30 min prior to the experiments. Then, each rat was gently placed in the light compartment of the apparatus. After 5 s, the guillotine door was opened, and the rat was allowed to enter the dark compartment. The latency with which each rat crossed into the dark compartment was recorded. The rats that waited more than 100 s to cross into the dark compartment were eliminated from the experiments. Once the rat crossed with all four paws into the next compartment, the guillotine door was closed and the rat was taken to its home cage. The acquisition trial was performed 30 min after the habituation trial. The rat was placed in the light compartment, and the guillotine door was opened 5 s later. As soon as the rat crossed into the dark compartment, the door was closed, and a foot shock (0.5 mA in intensity, 3 s) was immediately delivered to the grid floor of the dark room by an insulated stimulator.

One day after training, retention tests were performed to evaluate memory performance. Each rat was placed in the light compartment for 20 s, the door was opened, and the step-through latency for entering into the dark compartment was measured. The test session ended when the rat entered the dark compartment or remained in the light compartment for 300 s. No electric shock was applied during these sessions [28]. At the end of the experiments, rats were anesthetized with intraperitoneal injections of ketamine/xylazine (60 mg/kg and 6 mg/kg, respectively). Rats were sacrificed under anesthesia, and their brains were quickly removed, cleaned with chilled saline, and used for biochemical analysis.

2.5. Thiobarbituric acid reactive substance (TBARS) measurement

Malondialdehyde levels were measured as an index of lipid peroxidation. Malondialdehyde reacts with TBA as a thiobarbituric acid reactive substance (TBARS) to produce a red-colored complex with a peak absorbance at 535 nm [29]. Brains were homogenized with cold 1.5% KCl to make a 10% homogenate. Trichloroacetic acid–2-thiobarbituric acid–hydrochloric acid (2.0 ml) was added to 1.0 ml of the brain homogenate and mixed thoroughly. The solution was heated for 60 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 ×*g* for 10 min. The absorbance was determined at 535 nm against a blank that contained all the reagents except the sample. The amount of MDA equivalents formed was calculated using a molar extinction coefficient of 1.56×10^5 mol^{-1} cm^{-1} and expressed as nmol MDA equivalents/mg protein.

2.6. Total sulfhydryl groups assay

Total SH groups were measured using DTNB. This reagent reacts with SH groups to produce a yellow-colored complex with a peak absorbance at 412 nm [30]. Briefly, 1-ml Tris–EDTA buffer (pH = 8.6) was added to 50 μl brain homogenate in 2-ml cuvettes, and the sample absorbance was read at 412 nm against Tris–EDTA buffer alone (A). Next, 20 μl of DTNB (10 mM in methanol) was added to the mixture, and after 15 min (stored at laboratory temperature), the sample absorbance was read again (A1). The absorbance of the DTNB reagent was read as a blank (B) [31]. Total thiol concentration (mM) was calculated using the following equation: total thiol concentration (mM) = (A2 − A1 − B) × 1.07 / 0.05 × 13.6.

2.7. Data analysis

The data were analyzed using a one-way ANOVA and a post hoc Tukey's test for multiple comparisons. A level of $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Effects of quercetin pretreatment on kindling development

The effects of different doses of quercetin during kindling are shown in Fig. 1. The repeated application of 35-mg/kg PTZ induced behavioral seizures with increasing severity which culminated in clonic convulsions at the end of the kindling procedure. Pretreatment with different doses of quercetin (25, 50, and 100 mg/kg) before each kindling injection modified the stage of kindling development in a different manner. Pretreatment with 50 mg/kg of quercetin attenuated the seizure severity of the kindling procedure by lowering the mean seizure stages at the beginning of the kindling procedure compared to control ($P < 0.05$) (Fig. 1).

3.2. Effects of quercetin pretreatment on passive avoidance test

There was no significant difference between the different groups in the number of trials, thus confirming the uniformity of the groups. All animals reached the criteria during the training procedure. In the PTZ group, both the acquisition trials and memory retrieval

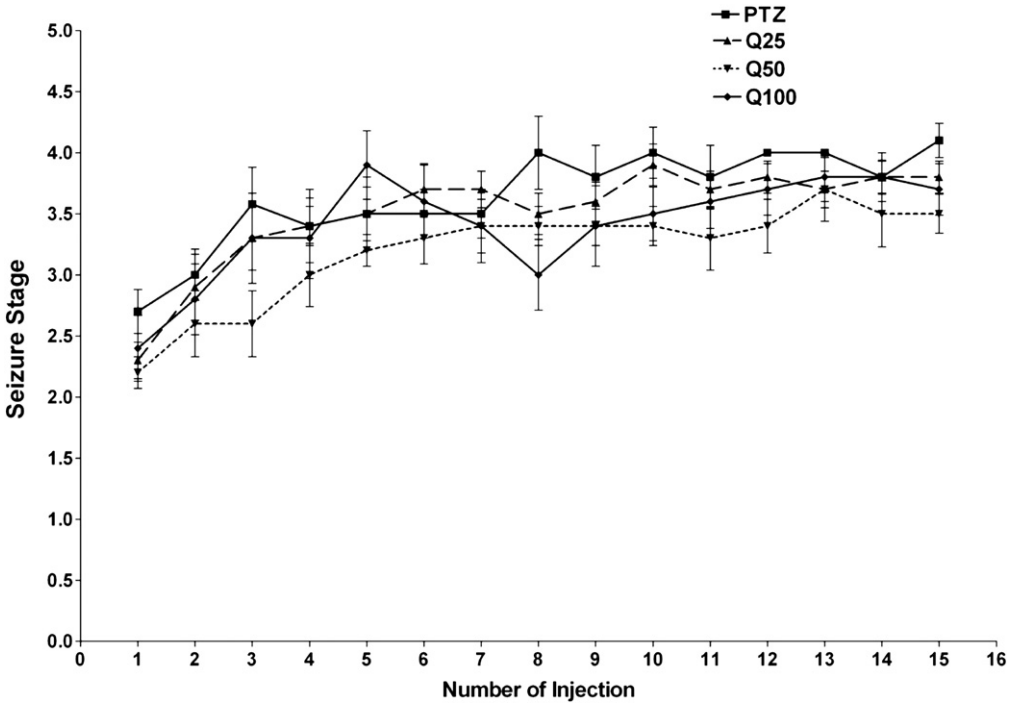

Fig. 1. The effects of repeated administration of different doses of quercetin (25, 50, and 100 mg/kg) on the development of PTZ-induced kindling (35 mg/kg, i.p., 15 injections total) compared to the control group. Data are expressed as mean seizure stage ± SEM (n = 10).

significantly decreased compared to the control group ($P < 0.05$) (Figs. 2A, B).

Quercetin at a dose of 50 mg/kg 30 min before the administration of PTZ every other day (35 mg/kg, i.p, 15 injections total) prior to training significantly increased acquisition trials and memory retrieval compared to the PTZ group ($P < 0.01$ and $P < 0.05$, respectively). This effect was not dose-dependent (Figs. 2A, B). Also, there were no significant differences in the acquisition trials and memory retrieval between the 50-mg/kg quercetin group and the control group.

3.3. Thiobarbituric acid reactive substance (TBARS) measurement

Malondialdehyde levels were increased following PTZ kindling in both the hippocampus and cerebral cortex in all groups (Fig. 3). However, MDA contents in the cortex were greater than those in the hippocampus, and there were more hippocampus MDA changes than cortex MDA changes.

Quercetin administration caused a significant elevation in free radical-mediated lipid peroxidation, as indicated by a significant increase in MDA levels at doses of 25, 50, and 100 mg/kg compared to the PTZ group in the hippocampus ($P < 0.001$, $P < 0.01$, and $P < 0.001$, respectively) (Fig. 3). Similarly, quercetin resulted in a significant increase in the MDA levels at doses of 25, 50, and 100 mg/kg compared to the PTZ group in the cerebral cortex ($P < 0.01$, $P < 0.05$, and $P < 0.01$, respectively) (Fig. 3).

However, in both tissues, the effect of quercetin on the elevation of MDA levels at the dose of 50 mg/kg was less than for the other doses of quercetin compared to the PTZ group.

3.4. Total sulfhydryl groups assay

Following PTZ kindling, quercetin did not cause significant elevation in total SH concentrations compared to the PTZ group in the hippocampus and cerebral cortex (Fig. 4). However, the elevation in total SH concentrations was dose-dependent in the cerebral cortex.

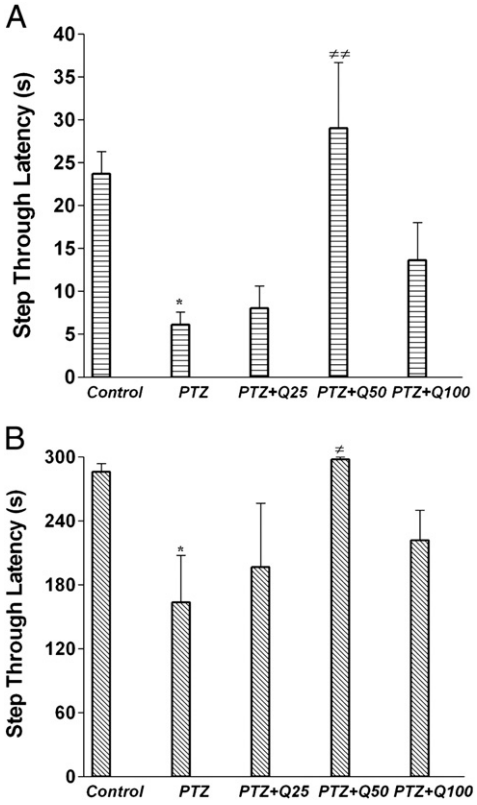

Fig. 2. Effects of different doses of quercetin (25, 50, and 100 mg/kg) on the step-through latencies in acquisition trials (A) and retention trials (B) in rats. The control group received PTZ injections every other day (35 mg/kg, i.p., 15 injections total) prior to the experiments. In the remaining three groups, 25, 50, or 100 mg/kg of quercetin was given 30 min before administration of PTZ every other day prior to the experiments. Retention tests were performed one day after training, Values are expressed as mean ± SEM. $^{*}P < 0.05$, compared to control, $^{\neq}P < 0.05$, $^{\neq\neq}P < 0.01$ compared to the PTZ group (n = 10, Tukey–Kramer test).

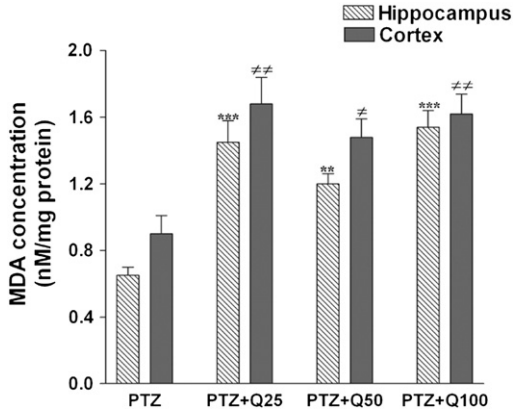

Fig. 3. Effect of quercetin on lipid peroxidation following PTZ kindling in hippocampus and cerebral cortex. Values are expressed as mean ± SEM. $^{**}P < 0.01$, $^{***}P < 0.001$ compared to the PTZ group in hippocampus samples. $^{\neq}P < 0.05$, $^{\neq\neq}P < 0.01$ compared to the PTZ group in cortex samples (n = 10, Tukey–Kramer test).

4. Discussion

In the present study, we investigated the possible effects of quercetin on oxidative stress and memory retrieval in kindled rats. Pretreatment with 50 mg/kg of quercetin attenuated seizure severity from the beginning of the kindling procedure by lowering the mean seizure stages compared to the control. However, this effect was not observed at both lower and higher doses.

It was established that in the PTZ kindling model, the susceptibility to seizures following injection of initially subconvulsant doses of PTZ was increased and culminated in GTCS. After repeated or single dose administration of PTZ, GABAergic function is decreased, and the stimulation and modification of sensitivities of different glutamate receptor subtypes in many brain regions occur [32,33]. With regard to the anticonvulsant effects of quercetin, it is possible that quercetin modulates $GABA_A$ receptors, and this has been reported for other flavonoids such as hispidulin at the specific dose range [34]. Thus, these protective effects of quercetin may be related to its modulatory effect on GABA receptors in this study.

On the other hand, it was reported that quercetin has proconvulsant activity [35]. It was also shown that the protective and toxic effects of quercetin depend on both timing and dose [36]. Similarly to our results, it was reported that quercetin has a narrow therapeutic dose range in in vitro studies. Thus, the risk for neurotoxicity is not negligible [21]. For this reason, it is possible that quercetin at a dose of 100 mg/kg did not show any anticonvulsant effects during kindling.

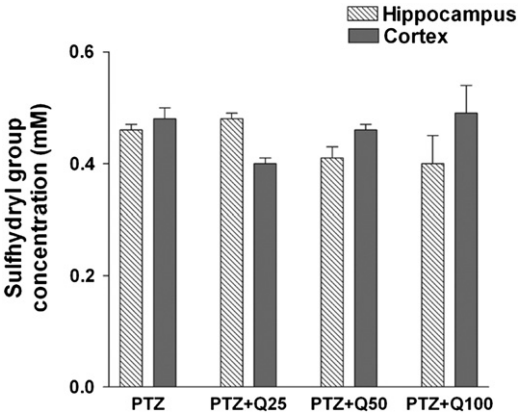

Fig. 4. Effect of quercetin on total thiol concentrations following PTZ kindling in the hippocampus and cerebral cortex. Values are expressed as mean ± SEM (n = 10, Tukey–Kramer test).

Antioxidants may have prooxidant and antioxidant activities [37]. Flavonoids are known to be antioxidants that can protect the cell from oxidative stress. However, at high concentrations, flavonoids and other polyphenols may be cytotoxic, causing increased mitochondrial permeability, cytochrome c release, caspase activation, increased levels of p53 and p21, suppression of Bcl-2, apoptosis induction, and necrotic cell death. They show prooxidant cytotoxicity in mammalian cells due to the formation of free radicals and oxidation products possessing quinone or quinomethide structure [38].

It has been established that quercetin also has prooxidant effects, and the observed concentration-dependent cytotoxic effect of quercetin may be related to the intracellular metabolic activation of quercetin to o-quinone [39]. Low concentrations of quercetin could stimulate cell proliferation and increase the total antioxidant capacity (TAC) of the cells, while higher concentrations decreased cell survival and viability, thiol content, TAC, and activities of superoxide dismutase, catalase, and glutathione *S*-transferase. Furthermore, quercetin decreased reactive oxygen species (ROS) production in cells but produced peroxides in the medium. Thus, it has been suggested that quercetin has complex oxidative and antioxidative effects [40]. Accordingly, it is possible that both the prooxidant and antioxidant effects of quercetin may be involved in the different effects of quercetin on the seizure threshold in kindled rats.

In this study, pretreatment with quercetin before administration of PTZ every other day and prior to training was followed by enhanced memory retrieval in rats. Quercetin 50 mg/kg significantly enhanced memory retrieval in the retention tests of a passive avoidance task compared to the control.

The direct role of flavonoids in memory acquisition, consolidation, and storage has been described previously in a study involving induced activation of neuronal signaling and gene expression in the brain. The results indicated that this may lead to changes in synaptic plasticity and neurogenesis in the brain, ultimately influencing memory, learning, and cognition [41].

Reactive oxygen species have been implicated in PTZ-induced seizures and kindling in brains lacking cerebella [33]. In addition, PTZ administration at a convulsant dose caused significant increases in whole brain fatty acid content in the rat hippocampus [42]. The activities of necessary rat brain metabolic enzymes changed in PTZ-kindled rats, and these changes occurred mainly in the frontal cortex [43].

In the current study, quercetin significantly increased MDA levels in the hippocampus and cerebral cortex following PTZ kindling in the quercetin groups compared to the PTZ group. However, this enhancing effect of quercetin at a dose of 50 mg/kg was less than for other doses. Furthermore, as our results show, it seems that the content of MDA changes between the quercetin groups in the hippocampus was more than that found in the cerebral cortex. In kindled animals with quercetin treatment, higher SH contents were not observed compared to the PTZ group.

Similarly, it was reported recently that MDA and protein carbonylation (PC) levels were higher in patients with epilepsy compared to a control group, and there was no difference in oxidation levels between untreated and treated patients [44]. In addition, patients with epilepsy treated with phenytoin and valproate had increased MDA levels [45,46]. It has also been shown that a quercetin-enriched diet results in increased levels of quercetin and its metabolites in the mouse brain. However, it did not change mRNA levels of antioxidants and genes relevant to Alzheimer's disease [47].

5. Conclusion

In our study, we observed that quercetin has anticonvulsant effects and enhanced memory retrieval in the passive avoidance task, but it also increased oxidative stress in kindled animals. Thus, quercetin could not be effective against oxidative stress in the hippocampus and cerebral cortex in kindled rats besides its anticonvulsant

effects and protection against memory impairment at a dose of 50 mg/kg. Further study is necessary to evaluate the molecular mechanisms of quercetin during PTZ kindling.

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